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## Proteases for Cleavage and Sequencing

Boehringer Mannheim I

Proteases continued

Product	Application	Specificity
<b>Thrombin</b> Coagulation factor IIa from human plasma lyophilizate EC 3.4.21.5	Coagulation research, medical research, protein structure analysis and biochemical research	Serine endopeptidase hydrolyzing peptide and ester bonds specifically at the carboxylic side of Arg.
<b>Trypsin</b> from bovine pancreas EC 3.4.21.4	Protein degradation and tissue dissociation	Serine endopeptidase hydrolyzing specifically proteins and peptides at the carboxylic side of the basic amino acids Arg and Lys. Amide and ester bonds of Arg and Lys are cleaved as well.

A free Poster "Products for Protein Biochemistry" is available on request from your local representative.

Tested for HBs antigen and for the presence of antibodies to HIV-1, HIV-2, HCV and found to be negative. See also page XIV.

## Proteases Sequencing grade

- ▷ Proteases, see pages 400 - 408
- ▷ Proteases and restriction proteases for the cleavage of fusion proteins, see pages 397 - 399
- ▷ Protease inhibitors, see pages 484 - 490
- ▷ Protease substrates, see pages 499 - 500
- ▷ Proteases for coagulation research, see page 588

Product	Application	Specificity
<b>Acylamino Acid Peptidase Sequencing Grade</b> from horse liver lyophilizate Acylamino-acid releasing enzyme EC 3.4.19.1	Deblocking of peptides for subsequent N-terminal sequence analysis	Exopeptidase releasing N-acyl amino acids from peptides and proteins: acyl-X-L-Y- (X=prefere- ntially Ser, Ala or Met; Y=unspecific amino acid). The specificity is tested with α-NMSH as substrate
<b>Carboxypeptidase P Sequencing Grade</b> from <i>Penicillium janthinellum</i> lyophilizate Peptidyl-L-amino-acid hydrolase EC 3.4.16.1	Protein structure and sequence analysis	Serine carboxypeptidase hydrolyzing amino acid residues (including Pro, Asp, Glu) from the C-termini of proteins and peptides. Release of Ser and Gly is considerably retarded
<b>Carboxypeptidase Y Sequencing Grade</b> from yeast lyophilizate Peptidyl-L-amino-acid hydrolase EC 3.4.16.1	Sequence analysis and limited hydrolysis of peptides and proteins, especially in combination with carboxypeptidases A and B	Serine carboxypeptidase hydrolyzing amino acids (including Pro) from the C-termini of proteins and peptides. High catalysis rate if the penultimate and/or terminal amino acid carries aromatic or aliphatic side chains. The release of Gly and Asp is considerably retarded. Dipeptides are completely resistant to cleavage
<b>Cathepsin C Sequencing Grade</b> from bovine spleen solution Dipeptidyl peptidase Dipeptidyl transferase EC 3.4.14.1	Processing of fusion proteins. Catalysis of dipeptide transfer (transamidation)	Cysteine protease catalyzing the successive removal of N-terminal dipeptides from polypep- tides. The reaction rate is dependent on the penultimate amino acid, whereby hydrophilic and hydrophobic residues are accepted. Degradation is blocked by N-terminal Lys or Arg. Pro as the second or third amino acid prevents also cleavage

To place an order: 0130-2228

0821/759-8545

Fax: 0821/759-8509

For technical inquiries: 0821/759-8529

# Protein Block

## Proteases for Cleavage and Sequencing

Characteristics	Inhibitors	Cat. No.	Pack Size
Specific activity: approx. 120 U/mg enzyme protein at 25°C with Chromozym® TH as substrate. Contaminants: < 3% factor Xa Molecular weight: approx. 33.6 kD pH-Optimum: 8.2-9.0	DFP, TLCK, PMSF, benzamidine, $\alpha_1$ -antitrypsin, $\alpha_2$ -macroglobulin, antithrombin III-heparin, hirudin and APMSF	602 400	20 U
Specific activity: approx. 110 U/mg lyophilizate at 25°C with Chromozym TRY® as substrate (approx. 40 U/mg lyophilizate at 25°C with BAEE as substrate). Formulation: salt-free lyophilizate Molecular weight: 23.5 kD pH-Optimum: 8.0	TLCK, DFP, PMSF, leupeptin, soybean trypsin inhibitor, trypsin inhibitor from hen egg, aprotinin, $\alpha_2$ -macroglobulin, $\alpha_1$ -antitrypsin, APMSF and antipain	109 819 109 827	500 mg 2 g

### Proteases Sequencing grade

Characteristics	Inhibitors	Cat. No.	Pack Size	Price
Purity: free of impurities which might interfere with the specific cleavage and/or with the separation of peptides in reversed phase HPLC Molecular weight: 60 kD pH-Optimum: 7.5-9.0	DFP	1 970 502	2 x 30 µg	596,-
Purity: free of impurities, which might interfere with amino acid analysis. 10 µg carboxypeptidase P sequencing grade contain < 10 pmol of each amino acid. Function and purity are checked by amino acid analysis and SDS-PAGE. Molecular weight: 51 kD pH-Optimum: 3.7-5.2	DFP, iodoacetic acid and p-mercuribenzoate	1 420 321 1 111 906	20 µg 3 x 20 µg	181,- 415,-
Purity: free of impurities, which might interfere with the amino acid analysis. 10 µg carboxypeptidase Y sequencing grade contain < 10 pmol of each amino acid. Function and purity are checked by amino acid analysis and SDS-PAGE. Molecular weight: 61 kD pH-Optimum: approx. 5.5 for acidic and 7.0 for basic amino acids	DFP, PMSF, ZPCK, 4-hydroxymercuribenzoate and aprotinin	1 420 348 1 111 914	20 µg 3 x 20 µg	181,- 415,-
Purity: highly pure, not standardized with albumin Molecular weight: 210 kD pH-Optimum: 4.0-6.0 for hydrolytic activity; 7.0-8.0 for transferase/transamidase activity	iodoacetate and formaldehyde	1 559 821 1 559 630	3 x 30 µg (3 x 30 µg) 3 x 250 µg (3 x 250 µg)	323,- 1 188,-

## Protein Biochemistry

# Proteases for Cleavage and Sequencing

### Proteases

- ➔ *Proteases sequencing grade, see pages 408 - 410*
- ➔ *Proteases for the cleavage of fusion proteins, see pages 397 - 399*
- ➔ *Protease inhibitors, see pages 484 - 490*
- ➔ *Protease substrates, see pages 489 - 500*
- ➔ *Proteases for coagulation research, see page 588*
- ➔ *Collagenases, dispases and trypsins for tissue dissociation, see pages 200 - 204*

Product	Application	Specificity
<b>Aminopeptidase M</b> from pig kidney suspension in ammonium sulfate solution $\alpha$ -Aminoacyl-peptide hydrolase (microsomal) EC 3.4.11.2	Study of protein sequences and identification of chemically modified amino acid residues in proteins	Metalloprotease, hydrolyzing completely peptides and proteins with a free $\alpha$ -amino group and L-amino acids. X-Pro bonds are not cleaved. The amino group of Asp, Gln or $\beta$ -Ala is not cleaved even after prolonged incubation
<b>Carboxypeptidase A</b> from bovine pancreas suspension in water Peptidyl-L-amino-acid hydrolase EC 3.4.17.1	Sequence analysis of proteins by successive cleavage of amino acids from the C-terminus of proteins	Zn-metalloprotease, catalyzing the release of C-terminal amino acid residues which possess a L-configuration and an unsubstituted $\alpha$ -amino group. Very slow release of Gly, Asp, Glu, Cys and CysSO <sub>3</sub> H, no release of Arg, Pro and hydroxyproline
<b>Carboxypeptidase B</b> from pig pancreas solution Peptidyl-L-lysine (-L-arginine) hydrolase EC 3.4.17.2	Sequence analysis of proteins by successive cleavage of basic amino acids from the C-terminus of proteins	Zn-metalloprotease catalyzing the hydrolysis of the basic amino acids L-Lys and L-Arg from the C-terminal position in polypeptides.
<b>Carboxypeptidase Y</b> from yeast lyophilizate Peptidyl-L-amino acid hydrolase EC 3.4.16.1 ➔ <i>Carboxypeptidase Y Sequencing Grade see page 408</i>	Sequence analysis and limited hydrolysis of peptides and proteins, especially in combination with carboxypeptidases A and B	Serine carboxypeptidase hydrolyzing L-amino acids (including Pro) from the C-termini of proteins and peptides. High catalysis rate, if the penultimate and/or terminal residue is an aromatic or aliphatic amino acid. The release of Gly and Asp is considerably retarded. Terminal Pro and $\beta$ -Ala are good substrates. Dipeptides are completely resistant to cleavage
<b>Chymotrypsin</b> $\alpha$ -Chymotrypsin from bovine pancreas salt-free lyophilizate EC 3.4.21.1 ➔ <i>Chymotrypsin Sequencing Grade see page 408</i>	Hydrolysis of proteins by chymotrypsin alone or in combination with other proteases. Suitable for peptide mapping, fingerprinting and sequence analysis	Serine endopeptidase, specifically hydrolyzing peptide bonds at the C-termini of Tyr, Phe and Trp. Leu, Met, Ala, Asp and Glu are cleaved at lower rates. Acts also upon amides and esters of susceptible amino acids and is used for peptide synthesis
<b>Elastase</b> from pig pancreas lyophilizate EC 3.4.21.36	Digestion of elastin; tissue dissociation together with collagenase and trypsin and solubilization of membrane proteins. Hydrolyzes furthermore fibrin, casein, denatured collagen (but no native collagen), albumin, proteins from soybean and various synthetic substrates. Cleaves preferentially adjacent to neutral amino acids	Serine endopeptidase, hydrolyzing peptide bonds at the C-terminal site of amino acids with uncharged non-aromatic side chains like Ala, Val, Leu, Ile, Gly and Ser.
<b>Endoprotease Arg-C</b> from mouse submaxillary glands lyophilizate ➔ <i>Endoprotease Arg-C Sequencing Grade see page 408</i>	Protein structure and sequence analysis	Serine protease, hydrolyzing specifically peptide and ester bonds at the carboxylic side of Arg.

# Protein Biochemistry

## Proteases for Cleavage and Sequencing

### Proteases

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Characteristics	Inhibitors	Cat. No.	Pack Size	Price
<p><b>Specific activity:</b> approx. 4 U/mg at 25°C (11 U/mg at 37°C) with leucine-4-nitranilide as substrate</p> <p><b>Molecular weight:</b> 280 kD (two identical subunits)</p> <p><b>pH-Optimum:</b> 7.0-7.5; up to 9.0, depending on the substrate concentration</p>	Leucinethiol, 2,2'-bipyridine and 1,10-phenanthroline	102 788	20 U (1 ml)	DM 148,-
<p><b>Specific activity:</b> approx. 35 U/mg at 25°C with hippuryl-L-phenylalanine as substrate</p> <p><b>Contaminants:</b> &lt; 0.1% chymotrypsin, &lt; 0.1% trypsin</p> <p><b>Molecular weight:</b> 34.5 kD</p> <p><b>pH-Optimum:</b> approx. 7.5, may vary with different substrates</p>	Chelating agents like pyrophosphate, oxalate, citrate, cysteine and 1,10-phenanthroline. Freezing and lyophilization inactivates the enzyme	103 225	25 mg (1 ml)	169,-
<p><b>Specific activity:</b> approx. 150 U/mg at 25°C with hippuryl-L-arginine as substrate</p> <p><b>Contaminants:</b> &lt; 5 mU chymotrypsin/mg protein, &lt; 0.7 mU trypsin/mg protein, &lt; 2% carboxypeptidase A. Potential chymotrypsin and trypsin activities are eliminated by DFP treatment.</p> <p><b>Stability:</b> a decrease in activity of approx. 10% may occur within 8 months</p> <p><b>Molecular weight:</b> 34.8 kD</p> <p><b>pH-Optimum:</b> 7.0-9.0</p>	Chelating agents and basic amino acids	103 233	5 mg (1 ml)	193,-
<p><b>Specific activity:</b> approx. 20 U/mg lyophilizate at 37°C with Z-Phe-Ala as substrate</p> <p><b>Stability:</b> a decrease in activity of approx. 10% may occur within 6 months</p> <p><b>Molecular weight:</b> 61 kD</p> <p><b>pH-Optimum:</b> approx. 5.5 for acidic and 7.0 for basic amino acids</p>	DFP, PMSF, ZPCK, 4-hydroxymercuri-benzoate and aprotinin	238 139	10 mg lyophilizate	380,-
<p><b>Specific activity:</b> approx. 90 U/mg lyophilizate at 25°C with acetyl-L-tyrosine ethyl ester as substrate</p> <p><b>Preparation:</b> from activated, crystallized chymotrypsinogen A</p> <p><b>Molecular weight:</b> 25 kD</p> <p><b>pH-Optimum:</b> 7.0-9.0</p>	Aprotinin, DFP, PMSF, phenothiazine-N-carbonyl chloride, TPCK, ZPCK, $\alpha_1$ -macroglobulin, $\alpha_1$ -antitrypsin, soybean trypsin inhibitor and chymostatin; not inhibited by APMSF	103 308 103 314	500 mg 1 g	58,- 82,-
<p><b>Specific activity:</b> approx. 130 U/mg protein (approx. 105 U/mg lyophilizate) at 25°C with N-acetyl-trialanyl-methyl ester as substrate</p> <p><b>Molecular weight:</b> 25.9 kD</p> <p><b>pH-Optimum:</b> 8.8</p>	DFP, $\alpha_1$ -antitrypsin, $\alpha_2$ -macroglobulin, 4-nitrophenol diethylphosphate, PMSF, 3,4-dichloroisocoumarin, elastatinal	1 027 891 1 027 905	10 mg 50 mg	148,- 465,-
<p><b>Specific activity:</b> approx. 220 U/mg protein (approx. 20 U/mg lyophilizate) at 25°C with N-tosyl-L-arginine-methylester as substrate</p> <p><b>Molecular weight:</b> 30 kD</p> <p><b>pH-Optimum:</b> 8.0-8.5</p>	DFP, $\alpha_2$ -macroglobulin and TLCK	289 590	100 U	211,-

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Art. 8444

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# Trypsin

(aus Schweinepankreas) lyophilisiert

gereinigt durch Affinitätschromatographie  
für die Proteinsequenzanalyse

enthält 3 x 50 µg

## Spezifikation

50 µg Lyophilisat enthalten ca. 2,5 Einheiten Trypsin (Insulin B<sub>28</sub>, 25 °C, 1 h, pH 8,5)

Abwesenheit von Chymotrypsin und anderen Fremdprotease-Aktivitäten nachgewiesen durch RP-HPLC mit Insulin B<sub>28</sub>

## Einheitendefinition

1 Einheit ist definiert als diejenige Enzym-Menge, die notwendig ist, um 1 mg Insulin B<sub>28</sub> in 1 Stunde bei 25 °C und pH 8,5 vollständig zu spalten.

## Spezifität

Trypsin für die Proteinsequenzanalyse spaltet bei pH 7,5 bis 9,0 spezifisch Peptidbindungen C-terminal an Lysin und Arginin.

## Stabilität

Stabil bei -20 °C, trocken gelagert. Eine Lösung (z.B. in sterilem, bidest. Wasser) ist bei -20 °C gelagert mindestens 4 Wochen ohne Einfluß auf die Spezifität stabil.

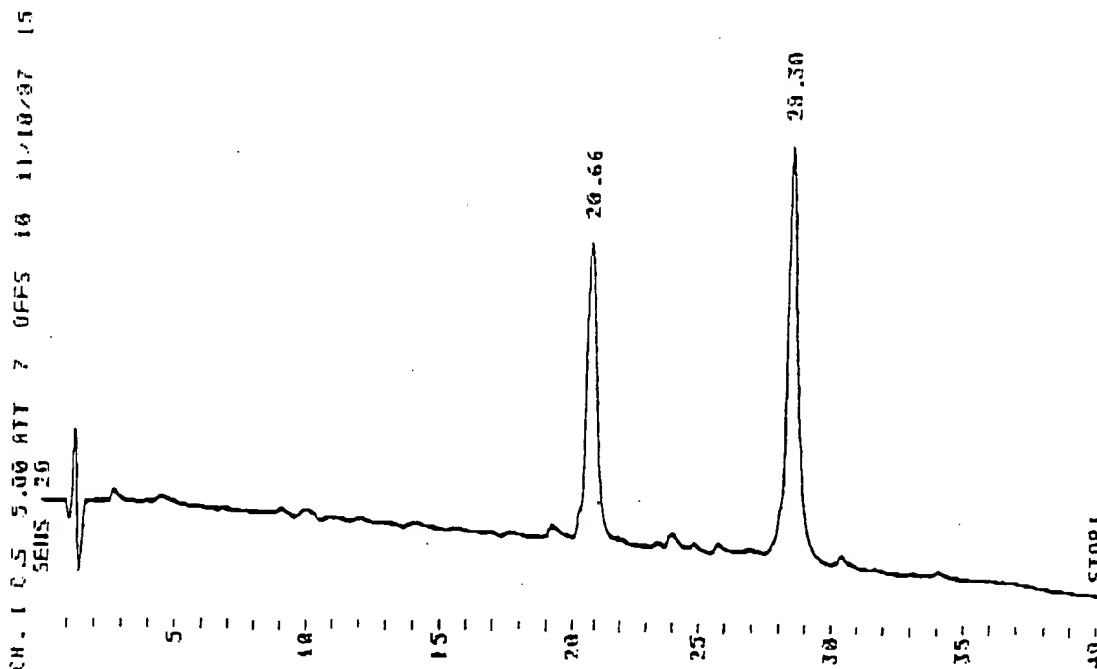
## Spezifitätstest

Die Überprüfung der Spezifität erfolgt durch Verdauung von Insulin B<sub>28</sub> und anschließender „reversed phase“ HPLC.

Verdauungsansatz: 0,09 mg Insulin B<sub>28</sub>  
0,05 mg Trypsin  
100 µl Tris/HCl-Puffer 50 mmol/l,  
pH 8,5

Inkubation 1 Stunde bei 25 °C

Chromatographie: Säule: LiChrospher 60 Select B  
(5 µm)  
Auftrag: 25 µl Verdauungsansatz  
Gradienten- 10% Acetonitril  
Lösung A: 90% TFA (0,1% v/v  
in Wasser)  
Gradienten- 90% Acetonitril  
Lösung B: 10% TFA (0,1% v/v  
in Wasser)  
Gradient: 35 min linear 0-35% B  
Durchflußgeschwindigkeit:  
1 ml/min  
Detektion: 215 nm





# Trypsin

sequencing grade

aus Rinderpankreas

4 x 100 µg - Best. Nr. 1047341

Handelstern: Lyophilisat, salzfrei.

*Trypsin sequencing grade* wird als spezifische Protease in hochreiner Form aus Rinderpankreas isoliert.

**Reinheit:** Das Enzym ist frei von Verunreinigungen, die im Trennbereich von Peptiden bei "reversed phase" HPLC (höchstempfindliche Detektion bei 208–230 nm) interferieren können. Funktions- und Reinheitskontrolle mittels HPLC bei jeder Charge garantieren gleichbleibende Qualität (Abb. 1).

**Spezifität:** *Trypsin sequencing grade* ist eine Serin-Protease. Sie spaltet bei pH = 7,5–9 spezifisch Peptidbindung C-terminal an Lysin und Arginin.

Die Spezifität von *Trypsin sequencing grade* wird mit Insulin, Kette B oxidiert (Insulin B<sub>ox</sub>) als Substrat überprüft. Dabei wird *Trypsin sequencing grade* in hoher Konzentration (1 Gewichtsteil *Trypsin sequencing grade* mit 18 Gewichtsteilen Insulin B<sub>ox</sub>) 18 Stunden inkubiert, um auch geringe Verunreinigungen durch Chymotrypsin zu erkennen (Abb. 2).

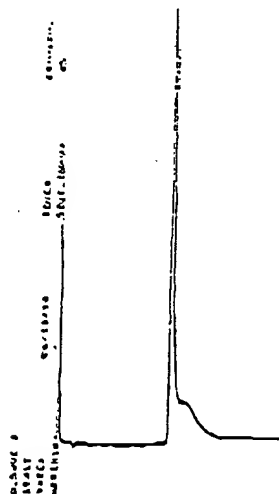


Abb. 1: Reinheit von *Trypsin sequencing grade* in der "reversed phase" HPLC.  
Menge: 20 µg *Trypsin sequencing grade*; Volumen: 20 µl; Säule: Aduagore RP 300 4,6x30 mm, 7 µm; Gradientenlösung A: Trifluoressigsäure (TFA), 0,1% (v/v) in Wasser; Gradientenlösung B: TFA, 0,1% (v/v) in Wasser; Acetonitril, 70% (v/v); Gradient: 30 min linear 0–100% B; Durchflussschwindigkeit: 0,5 ml/min; Wellenlänge: 215 nm.

**Hinweis:** Der Inhalt eines Röhrchens kann für mehrere gleichzeitige Ansätze verwendet werden. Bei Wiederholung sollte jedoch auf ein neues Röhrchen zurückgegriffen werden. Dadurch wird größtmögliche Sicherheit und Reproduzierbarkeit gewährleistet und eventuelle Kontamination oder Autolyse vermieden.

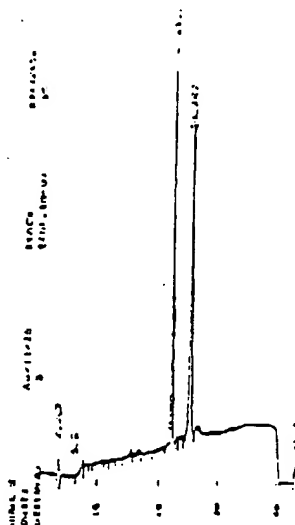


Abb. 2: Spezifität von *Trypsin sequencing grade* in der "reversed phase" HPLC.

Verdauungsansatz: Menge: 100 µg Insulin B<sub>ox</sub> + 3,6 µg *Trypsin sequencing grade* in 100 µl Tris-HCl-Puffer, 100 mmol/l, pH = 8,6; 18 Stunden bei 37 °C; "reversed phase" HPLC: 10 µl Verdauungsansatz mit Tris-HCl-Puffer auf 100 µl verdünnt; Säule: Polygasil C18; Gradientenlösung A: TFA, 0,1% (v/v) in Wasser; Gradientenlösung B: TFA, 0,1% (v/v) in Wasser; Acetonitril, 30% (v/v); Gradient: 30 min linear 0–100% B; Durchflussschwindigkeit: 1 ml/min; Wellenlänge: 215 nm.  
Spaltprodukte: 21,7 min Gly(23) – Lys(29),  
24,9 min Phe(1) – Arg(22).

Boehringer Mannheim Biochemica



**Stabilität:** Stabil bei +4 °C trocken gelagert. Eine Lösung in Trifluoressigsäure (TFA), 0,01% (v/v) oder HCl, 1 mmol/l, kann maximal 1 Woche bei +4 °C gelagert, verwendet werden.

**Anwendung:** Lyophilisiertes *Trypsin sequencing grade* in TFA, 0,01% (v/v), oder in HCl, 1 mmol/l, lösen. Zur Vermeidung von Autolyse darf die Inkubationstemperatur 37 °C nicht übersteigen. Die zu sequenzierenden Proteine werden im Verdauungspuffer (Tris-HCl, 100 mmol/l, pH = 8,5) gelöst. Bei schwer löslichen Proteinen sollte dem Puffer SDS, Harnstoff oder Guanidin-HCl in hoher Konzentration zugesetzt werden. Es wird empfohlen bei Verwendung von Harnstoff ebenfalls Methylamin, 20 mmol/l, zuzusetzen. Um eine für das Enzym tolerable Konzentration des Denaturierungsmittels im Verdauungsansatz zu erreichen, muß die Proteinlösung mit Puffer entsprechend verdünnt werden (Tabelle). Die empfohlene Enzymmenge beträgt 1/100 bis 1/20 der Gewichtsmenge an Protein; die Inkubationszeit sollte je nach Enzymmenge zwischen 2 und 18 Stunden bei 37 °C gewählt werden.

Denaturierungsmittel	Konzentration	Enzymaktivität in %
ohne Zusatz (Kontrolle)	—	100
Natriumdocecylsulfat (SDS)	0,001% (w/v)	120
	0,01 % (w/v)	110
	0,1 % (w/v)	105
Harnstoff	0,1 mol/l	86
	0,5 mol/l	86
	1,0 mol/l	90
Guanidin-HCl	0,1 mol/l	85
	0,5 mol/l	95
	1,0 mol/l	97
Acetonitril	1% (v/v)	100
	5% (v/v)	114
	10% (v/v)	124

Tabelle: Inkubation von *Trypsin sequencing grade* 200 µg/ml, mit verschiedenen Denaturierungsmitteln: 6 h bei 35 °C in Tris-HCl, 100 mmol/l, pH = 8,5. Aktivitätsbestimmung des *Trypsin sequencing grade* mit Chromozym<sup>®</sup> TRY.